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# CONTROLLED FLUID THERAPY

## With Hematocrit, Specific Gravity, and Plasma Protein Determinations

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WITH the increasing consciousness of the importance of fluids has come an increasing demand for more rapid, accurate, objective methods of determining the fluid requirements of acutely ill patients and for maintaining water balance when once established.

We have found that 4 relatively simple tests give much useful information concerning the state of hydration in seriously ill surgical patients (26). These are: (1) Determination of the percentage of cells in venous blood by means of a hematocrit; (2) determination of the specific gravity of the whole blood; (3) determination of the specific gravity of the plasma, and (4) calculation of the plasma protein content by means of a simple formula. The merit of these combined tests lies in the speed with which they can be done, the accuracy with which results can be reproduced, the small amount of equipment necessary, and the ease with which the technique may be mastered.

The danger lies in attempting to interpret these findings without a clear clinical picture of the patient. This approach to a difficult problem is presented as an aid to, not as a substitute for, already well established, diagnostic procedures.

The opinions expressed here have grown out of 3 years' experience. Because of the numerous requests as to both methods and interpretation (27, 28), each of the tests will be considered separately in some detail.

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### THE HEMATOCRIT

In 1885, Professor Blix presented at Upsala the first "haematokrit." It was modeled after the "laktokrit" used in the dairy industry. Employing this method, Hedin, in 1891, reported an average cell volume for adult males to be 48.0 per cent, and for adult females, 43.3 per cent. In the next 10 years there were many modifications. Capps, in 1903, introduced this work to America.

Haden (11), in 1923, popularized the large hematocrit tube in contradistinction to the capillary type and stressed the importance of using isotonic solutions of the various anticoagulants. Van Allen, in 1925, published a comprehensive résumé on the hematocrit method in experimental work. Wintrobe and Miller then introduced a 4 cubic centimeter graduate tube, made from a Mohr pipette; they employed potassium oxalate as the anticoagulant and allowed 6.7 per cent for cell

shrinkage. The publication of Haden (12), in 1930, is very complete and for further details this article is recommended.

In 1929, Sanford and Magath modified the Haden hematocrit. It is this tube which we prefer because it can be spun in any routine laboratory centrifuge, can be cleaned easily, and, being made of heavy glass, its durability is enhanced.

*Anticoagulant.* Heparin is the anticoagulant recommended for hematocrit determinations (8). It is an active fraction of the naturally occurring anticoagulant which was first isolated in Howell's laboratory by McLean in 1916. The heparin now employed is the sodium salt as prepared in the Connaught Laboratories of Toronto University, Canada. One milligram of the powder is sufficient anticoagulant for the blood in a Sanford-Magath hematocrit tube.

*Directions for taking blood and reading the hematocrit.* 1. Draw blood from vein with a sterile dry syringe without the use of a tourniquet if possible; or, if a tourniquet is used, release it and wait a minute for blood to recirculate before drawing the sample.

2. Gently introduce from 5 to 6 cubic centimeters into a Sanford-Magath hematocrit tube containing the proper amount of heparin. Avoid air bubbles.

3. Gently invert the tube two or three times to mix the blood with the anticoagulant.

4. Cork with rubber stopper (no-air No. 5), counter balance, and spin in centrifuge for 1 hour at 2,500 revolutions a minute (in emergency cases 15 minutes is sufficient).

5. Record the level of cells and the level of total blood sample.

6. To determine cell volume, divide the cells by the total volume. In normal blood the white cells form just a thin layer on top of the red cells, and it is not worth-while to attempt differentiation. In severe infections, however, usually associated with some degree of anemia, it is worth-while to measure the cell volume of the erythrocytes and leucocytes separately by dividing the volumes of each by the total blood volume in the sample.

7. Hemolysis in the plasma is most often caused by the use of a *wet syringe*, and less often by too vigorous shaking. The presence of a thin filament extending from bottom of the meniscus to top of the cells indicates a fibrinogen-fibrin mixture due either to insufficient anticoagulant or incomplete mixing. It decreases the cell volume somewhat and the plasma specific gravity determinations, but neither is significant for clinical purposes.

*Normal values* (Fig. 1). The normal cell volume values for the male range between 42 and 50 per cent, the average being approximately 46; while those for a female have a range of 39 to 43 per cent, with an average of 41.

### SPECIFIC GRAVITY

The earliest investigator of blood specific gravity was Robert Boyle who, in 1684, showed that both serum and whole blood were heavier than water. Jurin, in 1719, measured their weights more accurately and reported the specific gravity of the blood as 1.053 and that of the serum as 1.030. Sir John Davy, in 1839, determined by pycnometry the specific gravity of the whole blood and quoted freely from the earlier work of John Hunter who showed that specific gravity was high in the morning, high in inflammation, and high in dehydration.

Roy, in 1884, reported a simplified method of weighing blood. E. Lloyd Jones (16, 17), in 1887, and later in 1891, used this method and published observations which are still of outstanding value for specific gravity determinations in both health and disease.

Hammerschlag compiled a very complete summary and introduced the use of benzol and chloroform as a modification of the Roy method. Sherrington and Copeman observed that a fall in blood pressure during a long experiment or operation was accompanied by a fall in specific gravity of venous blood; hemorrhage was followed by a rapid fall; while vasoconstriction as seen in shock caused an early rise in the specific gravity of peripheral blood. Rogers showed the value of these tests in treating the severe dehydration of cholera.

In 1924, Barbour and Hamilton (2, 3, 4) presented a means for determining the specific gravity of body fluids which eliminated many disadvantages of the older methods (Fig. 2). The principle based on Stokes' law takes advantage of the fact that the time required for a drop of known volume to fall a fixed distance through an immiscible fluid is governed by the density of the drop and other factors, such as temperature, which can be controlled easily. It has been shown that differences of 0.2 of 1 per cent in weight are demonstrable, and that specific gravities may be reproduced with an accuracy of 0.0001.

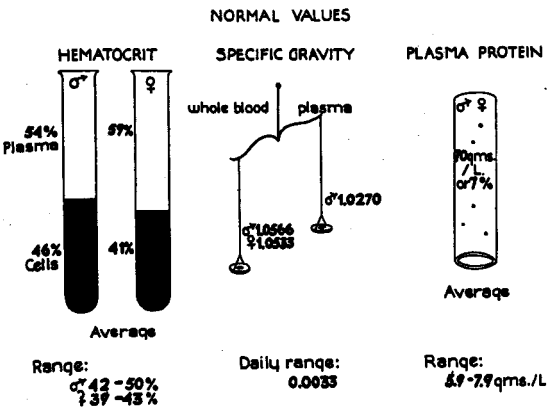


Fig. 1. Normal values.

Stebbins and Leake, in 1927, and later Polowe, and Bender and Polowe employed this method for clinical purposes. Guthrie, in 1932, reported that when compared with other methods for evaluation of blood conditions it stood first from every standpoint. For those unacquainted with the values of whole blood specific gravity, he offered the following simplified comparative table of approximate values:

| Specific gravity | Hemoglobin | Hematocrit per cent cells | Red blood count |
|---|---|---|---|
| 1.030 | 0 | 0 | 0 |
| 1.035 | 20 | 10 | 1,000,000 |
| 1.040 | 40 | 20 | 2,000,000 |
| 1.045 | 60 | 30 | 3,000,000 |
| 1.050 | 80 | 40 | 4,000,000 |
| 1.055 | 100 | 50 | 5,000,000 |
| 1.060 | 120 | 60 | 6,000,000 |

It is a modification of the Barbour and Hamilton apparatus[1] which we use routinely in the determination of whole blood, serum, and plasma specific gravity.

*Normal values.* In the male the average value of peripheral blood is 1.0566 and in the female 1.0533. A swing of 0.0033 occurs daily; the blood is more concentrated in the morning.

*Practical directions for determination of the specific gravity of whole blood* (Fig. 3). 1. Observe and record temperature of water bath in which the falling drop tubes are immersed.

[1]Complete sets may be obtained from Eimer and Amend, New York City, or LaMotte Chemical Company, Baltimore, Maryland.

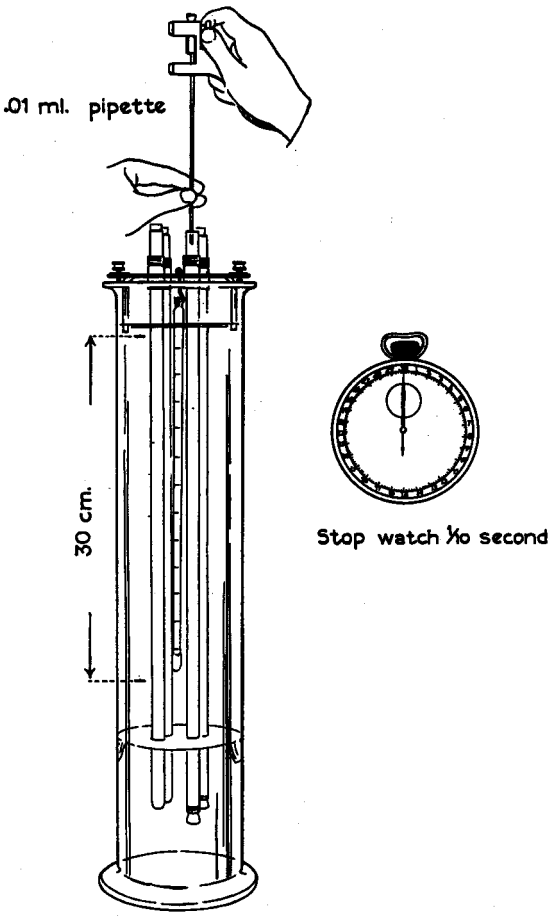


Fig. 2. Modified Barbour and Hamilton falling drop apparatus with Guthrie pipette holder. From the graduated pipette 0.01 ml. of plasma is released into a tube containing a mixture of bromobenzene and xylene and timed with a stop watch in its course between the two marks on the tube. The same process is carried out with a solution of known specific gravity and the weight of the unknown calculated (4).

2. Set stop watch at zero time.
3. Remove cork from the bromobenzene tube to be used.
   $B_1$ for normal and concentrated bloods (6).
   Specific gravity 1.0530.
   $B_2$ for anemic bloods.
   Specific gravity 1.0430.
4. Load pipette into holder.
5. Puncture finger; wipe away first drop; draw up blood into the calibrated pipette from a free bleeding, puncture wound to the second mark. (The pipette is made to deliver 2 drops of .01 milliliter each.)
6. Quickly (for no anticoagulant is used with whole blood) wipe the outside of the pipette with gauze, bring the drop of blood even with the middle

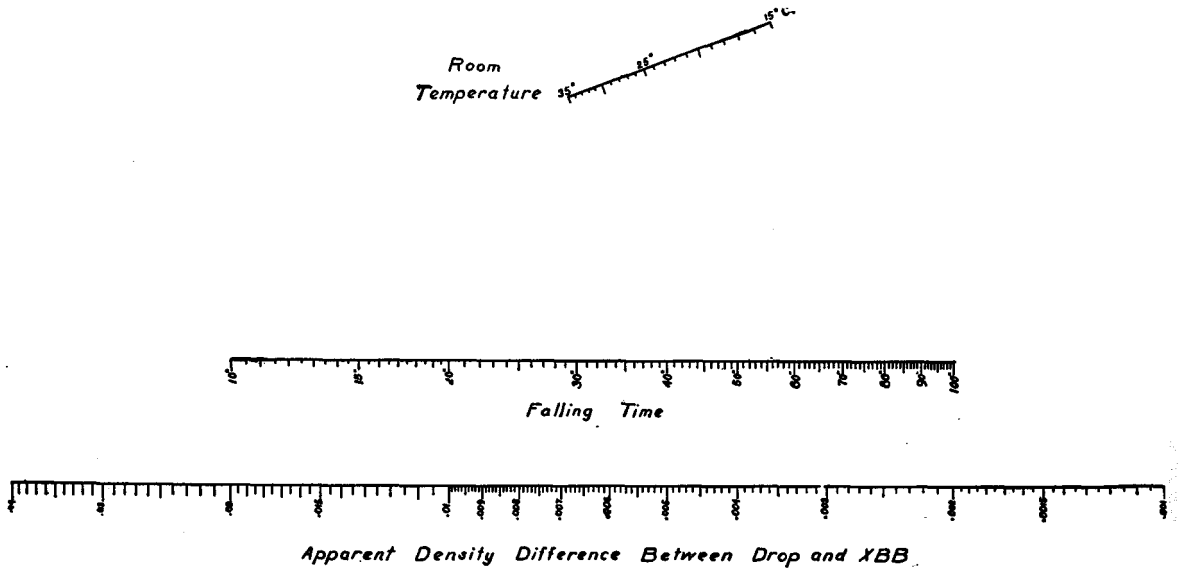


Fig. 3. Nomogram. Directions: (1) Insert pin with thread attached on the "temperature scale" at the recorded temperature of the water bath; (2) draw thread across the "falling time" scale at the correct time in seconds that it took the plasma to fall 30 centimeters; (3) read off the figure which the thread bisects on the *apparent density difference* scale. (This gives the difference in density between the xylene bromobenzene mixture and the plasma); (4) repeat same steps for the standard; (5) calculate specific gravity as directed. (Courtesy of H. G. Barbour, J. Am. M. Ass., 1927, 88: 91–94.)

mark on the pipette, insert the tip of the pipette just below the surface of the bromobenzene xylene mixture, with care not to touch the sides of the tube now turn the thumb screw on the pipette holder until the blood is at the level of the next mark, then gently lift the pipette out of the tube, and release the drop of blood. The surface tension existing between the blood and the mixture in the tube causes its release.

7. Time the fall of the drop of blood by pressing the stop watch as the center of the drop passes the upper mark and again as it passes the lower mark, 30 centimeters below on the tube, and record the falling time.

8. Repeat the procedure using another finger if the blood is not flowing freely from the first puncture, and a clean pipette. Two drops of whole blood should not be dropped from the same pipette, for the second drop is always heavier as the result of sedimentation of the cells in the interval during which the first drop is falling. Pipettes should be rinsed with ammonia water immediately after they have been used for whole blood specific gravity, then washed with water, alcohol, and ether. Record the time.

Cleanliness of these capillary tubes is the greatest limiting factor for securing accurate results. Redistilled acetone can be employed to advantage in carrying out this technique.

9. In a similar manner draw up the standard[1] just above the upper mark on the pipette, draw it down even to the mark by touching the tip to a piece of gauze, then determine its falling time between the 2 marks on the tube.

*Criteria for checks on falling time.* When a drop of blood, plasma, serum, or standard potassium sulphate solution falls in the bromobenzene mixture, successive drops from the same or different pipettes should give apparent density differences of not greater than $1 \times 10^{-4}$. It will be observed (Fig. 3) that 0.1 of a second in the rapid range of falling time constitutes a larger range in the apparent density differences than 0.5 of a second difference in the falling time of 2 successive drops at the other end of the scale. When accurate checks can not be made, a sufficient number of drops should be done to establish a good average falling time.

*Reasons for inability to get checks on drops.* (1) Dirty pipettes cause drops of varying sizes to be delivered. (2) Slipping drop due to rubber tubing in pipette holder being too large causing incomplete suction. (3) Too much tension on thumb screw at moment of release causes rebound of fluid in pipette when drop is released. Thumb screw should compress rubber tubing in holder uniformly and should be released before pipette is removed from tube so that level of fluid in pipette remains at the mark. (4) Torsion of hand steadying tip of pipette increases

[1] *The standards should be preserved under oil;* small portions being removed for daily work.

pressure in rubber tube and, therefore, a rebound suction action occurs at release. Relieve all tension before drop is released. (5) Currents in the dropping tube due to rapidly changing temperature on one side of the water jacket, e.g., that caused by opening a window or lighting a burner near the apparatus. (6) Air bubble or particle of solid material. (7) Poor reaction time in using stop watch. A stop watch stand[1] will aid in accurate timing. (8) Incomplete mixture of the bromobenzene xylene solution causing varying rates of speed in the falling drop. (9) Gross differences in temperature between bromobenzene mixture and plasma. These are minor technical details which are quickly mastered but which in the beginning give trouble.

*Determination of the specific gravity.* 1. To calculate the apparent density difference between the blood and the mixture through which it falls, insert a pin on the temperature scale (Fig. 3) at the temperature of the water bath and with a thread cross the "falling time" scale at the figure given by the stop watch. The thread will cross the "apparent density difference" line at a point which gives the difference in density.

2. Calculate the apparent density difference between the standard and the bromobenzene xylene mixture in a similar manner.

3. Calculate the true density difference between the blood and the standard by subtracting the lesser figure from the greater.

4. Correct for temperature by subtracting from the specific gravity of the standard 0.0001 for each 2 degrees' change in temperature above 20 degrees C. and by adding 0.0001 for every 2 degrees below 20 degrees C.

5. If the blood falls faster than the standard, it is heavier; therefore, the true density difference is added to the corrected specific gravity of the standard. If the blood falls slower than the standard, it is lighter; therefore, the true density difference is subtracted.

Example:

| | | |
|---|---|---|
| Specific gravity of standard. | 1.0550 | |
| Correction for temperature of 22° C. | −0.0001 | |
| Corrected specific gravity of standard | | 1.0549 |
| Falling time of blood | 21.5 secs. | |
| Apparent density difference. | 0.0115 | |
| Falling time of standard | 27.5 secs. | |
| Apparent density difference. | 0.0086 | |
| True density difference 0.0115−0.0086 | | 0.0029 |
| Specific gravity of blood | | 1.0578 |

This whole procedure can be carried out in about 2 minutes. This cannot be used to determine proteins. While the proteins do contribute to the specific gravity of whole blood, the red blood cells and their hemoglobin content are more significant because of their greater weight.

*Specific gravity of plasma.* The steps for determining specific gravity of plasma are exactly the same as those described for whole blood, except that 2 drops are used from each pipette and No. 3 standard (specific gravity 1.0268). The determination is easier because there is no tendency toward coagulation.

For heavy plasma tube $P_1$ is used (specific gravity 1.0230); for lighter plasma tube $P_2$ (specific gravity 1.0130). Again the falling time of the plasma is checked against the falling time of the standard and the true density difference is added or subtracted to the corrected specific gravity of the standard.

It is this plasma specific gravity from which the proteins are calculated. It is the plasma specific gravity test which is routinely done on all pre-operative cases; the whole blood specific gravity is determined only in those cases in which difficulty is anticipated or when the course of the patient is being followed at the bedside during emergency treatment.

## PROTEINS

In 1927, Atchley and Benedict, after a careful study of the electrolyte distribution in a case of severe intestinal obstruction, suggested that a simple determination of serum protein content might be the best aid in following the degree of dehydration and treatment.

In 1929, Moore and Van Slyke showed that there is a constant relationship between the specific gravity of the serum or plasma and the protein content. For plasma they expressed this relationship by the formula: $P=343(G-1.0070)$, in which P equals the grams of protein per 100 cubic centimeters of plasma and G equals the specific gravity of the plasma. This work was done on human plasma and the maximum deviation was found to be 0.6 gram per cent.

Weech, Reeves, and Goettsch, in 1936, checked the work of Moore and Van Slyke. In their studies specific gravities were determined by pycnometry and nitrogen determinations by the micro-Kjeldahl method. Their formula for plasma was given as $P=340.1(G-1.00687)\pm 0.103$. It is this formula which we have routinely used.

We have rechecked Weech's formulas for both serum and plasma using a series of unselected clinical cases, and have found it to be

[1] May be obtained from A. R. and J. E. Meyland, New York City

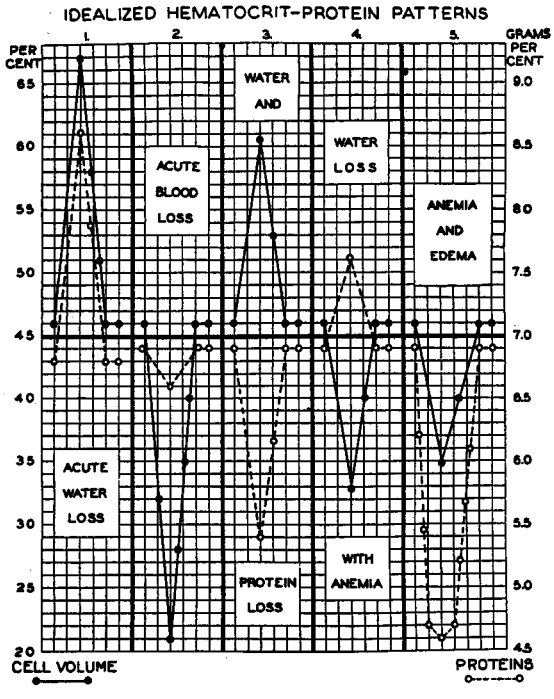


Fig. 4. These patterns represent idealized, superimposed, graphic histories of the changes in cell volumes and plasma protein determinations on several cases of each type with the time element eliminated for the sake of brevity in comparative representations. Each curve begins at a hematocrit reading just above the normal heavy black line; the proteins just below the line and each returns to the beginning position.

Column 1. Acute water loss. Here there is marked hemoconcentration and dehydration as indicated by the rise in both cell volume and protein level. Such a pattern is typical of conditions in which there is excessive vomiting, severe diarrhea, diminished fluid intake, or uncomplicated shock.

Column 2. Acute blood loss. Hemorrhage gives a picture of steadily falling hematocrit reading and moderate protein loss.

Column 3. Water and protein loss. This is the picture so characteristic of severe burns, to a slightly less degree in ruptured peptic ulcers, ruptured appendices with peritonitis, and persistent fistulas from the bowel.

Column 4. Water loss with anemia. Relative hyperproteinemia in the presence of secondary anemia of chronic illness is a fairly reliable index of the degree of dehydration.

Column 5. Anemia and edema. All values are below the normal line. When proteins reach a concentration of less than 5.5 grams per cent functional disturbances related to the water content of the tissue may be expected; when below 5.0, true edema usually supervenes.

very accurate when the protein percentages, as calculated from the specific gravity determinations, were checked by the standard micro-Kjeldahl method for nitrogen determination.[1] Comparing the mean average between the determined and calculated protein values in one series, we found that the difference amounted to ±0.16 gram per cent or 2.3 per cent.

The essence of the relationship is that plasma, completely free of protein, has a specific gravity of about 1.00687. Only rather large changes in the salt content of the blood upset this constant. The factor of 340.1 indicates that for each increase of 1 gram per cent of protein the specific gravity rises 1/340 or .00294; in other words, each increase in the specific gravity of 0.0001 indicates 0.03 gram per cent increase in protein.

By this method the total protein content values are open to question in several types of cases that have come to light so far. They are: gross hemolysis, severe diabetes, hypercholesterolemia, gross lipemia, and excessive bilirubinemia.

To facilitate the rapid calculation of the total protein content of the plasma from the plasma specific gravity determinations, a chart with comparative values already worked out has proved of assistance. The following is a condensed form of such a chart:

| Specific gravity | Protein Grams per cent | Specific gravity | Protein Grams per cent |
|---|---|---|---|
| 1.0187 | 4.01 | 1.0275 | 7.01 |
| 1.0202 | 4.52 | 1.0290 | 7.52 |
| 1.0217 | 5.03 | 1.0304 | 7.99 |
| 1.0231 | 5.51 | 1.0319 | 8.50 |
| 1.0246 | 6.02 | 1.0334 | 9.01 |
| 1.0260 | 6.50 | 1.0348 | 9.49 |

## INTERPRETATION OF STUDY

A fairly definite idea of the history and clinical picture of the patient should always be sought before a final evaluation of the state of hydration is made from the data given by the preceding tests. Most important is the trend toward or away from normal as judged by repeated tests and not a single set of determinations. Certain well defined patterns have recurred many times in following a large series of cases, and these have proved of great aid in the interpretation of the values in any specific case.

[1]In the medical and surgical laboratories through the kindness of Dr. Dana W. Atchley and Dr. Louis Bauman.

In *simple dehydration* (Fig. 4, column 1), whether from lack of fluid intake, diarrhea, excessive sweating, severe vomiting, or shock of a psychogenic, traumatic, or postoperative origin uncomplicated by hemorrhage, there is a rise in the cell volume, the whole blood and plasma specific gravity, and the plasma protein percentage. In the first 3 of these conditions, treatment consists in administering fluids until these elevated values tend to approach normal. In shock, however, the mechanism is more complicated; hence treatment, to be rational, must attempt to overcome first the severe arteriolar and venular spasm, second, the capillary paralysis and dilatation, and third, the great loss of circulating blood volume. Hypertonic sodium chloride is particularly effective in relieving this arteriolar spasm, in decreasing the viscosity of the blood, and in aiding the return of fluid from the tissues into circulation. It should be used cautiously in collapse due to dehydration (26). Suprarenal cortical hormone (eschatin) has proved valuable in restoring capillary tone, raising the blood pressure, and redistributing electrolytes. To maintain any gain initiated by the sodium chloride and eschatin therapy, blood transfusions are given as dictated by the findings on repeated blood studies.

In *hemorrhage* (Fig. 4, column 2), either obvious or concealed, there is an immediate fall in the specific gravity of the whole blood and a drop in the cell volume as determined by the hematocrit. The plasma specific gravity changes are less marked (26). Even in severe hemorrhage, these values may not be abnormal due to readjustments of plasma proteins and circulating volume. They do not approach the percentage loss of cellular elements as indicated by the fall in whole blood specific gravity and the hematocrit.

Treatment here consists of restoring blood volume by transfusions. It is of utmost importance in cases of shock when the patient is unconscious to determine whether that shock is complicated by hemorrhage or not. These simple tests can make this differentiation in the vast majority of cases.

When there is not only loss of fluid but loss of protein as well (Fig. 4, column 3), there is a tendency for the hematocrit curve to rise while the protein values continue to fall. Such patterns are typical of severe burns, ruptured peptic ulcers with peritonitis, and even ruptured appendices with large abscess formation as the result of a great pouring out into the peritoneal cavity of an exudate rich in protein. In these cases, the problem of water balance is doubly hard. The extreme hemoconcentration and shock, if present, must be combated by means of adequate fluid administration; yet, the already lowered protein concentration must not be reduced to the edema level which in most patients is reached at about 5 grams per 100 cubic centimeters of plasma. Ravdin and his colleagues have laid great stress on the part that such hypoproteinemia plays in the malfunctioning of tissues and organs, particularly after traumatic or operative insult.

*When acute changes take place in chronic disease* (Fig. 4, column 4) in which there already exists an anemia and probably a hypoproteinemia, any one of the ordinary tests, i.e., red cell count, hemoglobin, or hematocrit determination, will not evaluate the true state of hydration. A combination of all these tests is necessary, in particular, the determination of plasma specific gravity. A sudden water loss is reflected by an increase in the weight of plasma before other changes become apparent. This resultant hyperproteinemia is relative. Treatment consists in reducing the protein values to approximately normal levels and then restoring the cellular elements of the blood by appropriate means.

*Impending edema* (Fig 4, column 5) may be suspected by a gradually falling plasma protein level. Such water logging of tissues is detrimental in any surgical condition. In severe burns local edema causes an early creeping of the tan, allowing infection at its periphery. In postoperative resections or anastomoses, the stomas become swollen and obstructed, the motility of the gut is lessened, and the tendency for ileus increases. A definite relationship exists between the water content of tissues and the healing of wounds, particularly in those cases in which catgut has been used as the suture material and its tensile strength is affected. Repeated determinations of the plasma protein content by an

easy but accurate method assist in the prevention of such complications.

There is no formula at present which will state in a simple manner the amount of fluid necessary to rehydrate any given dehydrated patient, nor is there any rule of thumb for the type of fluid to be used in each case. Certain precautions seem wise; e.g., in cases of hemoconcentration in which the hematocrit reading shows over 60 per cent cell volume, it seems unwise to use large quantities of hypertonic solutions because of the danger of bringing into circulation cell water which is rich in electrolytes definitely toxic when present in the plasma in quantities greater than normal (26).

In the severely ill there may be a complete loss of the ability to utilize fluids of any type in almost any quantities until the severe spasm of the peripheral vessels is relieved and the consequent return to the circulation of the sequestrated blood in the paralyzed peripheral capillaries.

The only safe way at present is to measure the degree of hemoconcentration or anemia, the degree of dehydration or edema, institute the therapy suggested by the findings, follow the curve of progress by repeated determinations, and evaluate the state of hydration day by day or hour by hour if need be.

SUMMARY

1. Four simple tests are presented in some detail which may be used as emergency measures in determining and regulating the state of hydration of acutely ill persons. They are: (1) the determination of the cell volume of venous blood by means of the hematocrit; (2) determination of the specific gravity of the whole blood by the modified Barbour and Hamilton method; (3) determination of the specific gravity of the plasma; (4) calculation of the plasma proteins from the plasma specific gravity by a simple formula.

2. By means of the data thus acquired one may determine degrees of water loss, water plus protein loss, anticipate the onset of shock, differentiate shock due to simple circulatory collapse from shock complicated by hemorrhage, detect dehydration in the presence of anemia, predict the approach of an edema level of proteins, and direct treatment more rationally for the alleviation of any of these conditions.

REFERENCES

1. Atchley, D. W., and Benedict, E. M. The distribution of electrolytes in intestinal obstruction. J. Biol. Chem., 1927, 75: 697–702.
2. Barbour, H. G., and Hamilton, W. F. Blood specific gravity: its significance and a new method for its determination. Am. J. Physiol., 1924, 69: 654–661.
3. Idem. The falling drop method for determining specific gravity. J. Biol. Chem., 1926, 69: 625–640.
4. Idem. The falling drop method for determining specific gravity. Some clinical applications. J. Am. M. Ass., 1927, 88: 91–94.
5. Bender, T., and Polowe, D. The specific gravity of the blood during spinal anesthesia. J. Lab. & Clin. Med., 1932, 18: 275–281.
6. Boyle, R. Memoirs for the Natural History of Human Blood, Especially the Spirit of That Liquor. London: S. Smith, 1684.
7. Capps, J. A. A study of volume index. Observations upon the volume of erythrocytes in various disease conditions. J. Med. Research, 1903, 10: 367–401.
8. Charles, A. F., and Scott, D. A. Studies on heparin IV. Observations on the chemistry of heparin. Bio-Chem. J., 1936, 30: 1927–1933.
9. Davy, J. Researches, Physiological and Anatomical. Vol. 2. London: Smith, Elder & Co., 1839.
10. Guthrie, C. C. An apparatus for quickly measuring the specific gravity of body fluids. J. Lab. & Clin. Med., 1932, 17: 1158–1162.
11. Haden, R. L. The Volume and Hemoglobin Content of the Erythrocytes in Health and Disease. Folia haematol., 1925, 31: 113–135.
12. Idem. The technic of determination of the relative mass, the individual cell volume, and the volume index of the erythrocytes of man. J. Lab. & Clin. Med., 1930, 15: 736–746.
13. Hammerschlag, A. Ueber das Verhalten des specifischen Gewichtes des Blutes in Krankheiten. Centralbl. f. klin. Med., 1891, 12: 825–837.
14. Hedin, S. G. Der Haematokrit, ein neuer Apparat zur Untersuchung des Blutes. Skandin. Arch. f. Physiol., 1891, 2: 134–140.
15. Hunter, J. A Treatise on the Blood, Inflammation, and Gun-shot Wounds. London: Sherwood, Gilbert & Piper, 1828.
16. Jones, E. L. On the variations in the specific gravity of the blood in health. J. Physiol., 1887, 8: 1–14.
17. Idem. Further observations on the specific gravity of the blood in health and disease. J. Physiol., 1891, 12: 299–346.
18. Jurin, J. Philosophical Transactions of the Royal Society of London, 1719, 5: 326–333.
19. McLean, J. The thromboplastic action of cephalin. Am. J. Physiol., 1916, 41: 250–257.
20. Moore, N. S., and Van Slyke, D. D. The relationships between plasma specific gravity, plasma protein content and edema in nephritis. J. Clin. Invest., 1930, 8: 337–355.
21. Polowe, D. The specific gravity of the blood in pregnancy and in the puerperium. Am. J. Obst. & Gynec., 1932, 23: 843–849.
22. Ravdin, I. S. Some recent advances in surgical therapeusis. Ann. Surg., 1939, 109: 321–333.
23. Rogers, L. Cholera and Its Treatment. London: Henry Frowde, Oxford University Press, 1913.

24. ROY, C. S. Note on a method of measuring specific gravity of the blood for clinical use. J. Physiol., Proc. Physiol. Soc., 1884–1885, 5: ix–xi.
25. SANFORD, A. H., and MAGATH, T. B. A new centrifuge tube for volume index determinations (modified Haden method). J. Lab. & Clin. Med., 1929, 15: 172.
26. SCUDDER, J. Shock: Blood Studies as a Guide to Therapy. Philadelphia: J. B. Lippincott Co., 1940.
27. SCUDDER, J., DREW, C. R., and SLOAN, L. W. Anhydremia in appendicitis. Surg. Clin. N. America, 1939, 19: 295–306.
28. SCUDDER, J., ZWEMER, R. L., and WHIPPLE, A. O. Acute intestinal obstruction, evaluation of results in 2150 cases; with detailed studies of 25 showing potassium as a toxic factor. Ann. Surg., 1938, 107: 161–197.
29. SHERRINGTON, C. S., and COPEMAN, S. M. Variations experimentally produced in the specific gravity of the blood. J. Physiol., 1893, 14: 52–96.
30. STEBBINS, G. E., and LEAKE, D. C. Diurnal variations in blood specific gravity in normal dogs and humans. Am. J. Physiol., 1927, 80: 639–642.
31. VAN ALLEN, C. M. An hematocrit method. J. Lab. & Clin. Med., 1925, 10: 1027–1040.
32. WEECH, A. A., REEVES, E. B., and GOETTSCH, E. The relationship between specific gravity and protein content in plasma, serum, and transudate from dogs. J. Biol. Chem., 1936, 113: 167–174.
33. WINTROBE, M. M., and MILLER, M. W. Normal blood determinations in the south. Arch. Int. Med., 1929, 43: 96–113.